

(i) an attenuated live mutant bacterium having a genome wherein a native gene having a function of ferric uptake regulation (*fur* gene) has been modified by mutation whereby expression of a gene product corresponding to said *fur* gene is regulated independently of the iron concentration in the environment of the bacterium; and

(ii) a non-viable preparation comprising bacterial membrane antigens from cultured cells of a mutant bacterium having a genome wherein a native gene having a function of ferric uptake regulation (*fur* gene) has been modified by mutation whereby expression of a gene product corresponding to said *fur* gene is regulated independently of the iron concentration in the environment of the bacterium;

together with:-

(b) a pharmaceutically acceptable diluent or carrier.

26. The vaccine composition of claim 25, wherein said mutant bacterium comprises *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Helicobacter pylori*, *Salmonella typhi*, *Salmonella typhimurium*, or *E. coli*.

27. The vaccine composition of claim 25, wherein said non-viable preparation comprising bacterial membrane antigens is obtained by isolating bacterial membrane vesicles from said cultured cells of said mutant bacterium.

28. An attenuated mutant bacterium having a genome wherein a native *fur* gene, having a function of ferric uptake regulation, has been modified by mutation whereby expression of a gene product corresponding to said *fur* gene is regulated independently of the iron concentration in the environment of the bacterium.

29. The attenuated mutant bacterium of claim 28 which is a gram-negative bacterium.

30. The attenuated mutant bacterium of claim 28, wherein the mutant bacterium comprises a *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Helicobacter pylori*, *Salmonella typhi*, *Salmonella typhimurium*, enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), verotoxigenic *E. coli* (VTEC), *Vibrio cholerae*, *Shigella spp.*, *Haemophilus influenzae*, *Bordetella pertussis* or *Pseudomonas aeruginosa* species.

31. The attenuated mutant bacterium of claim 28, wherein the mutant bacterium comprises a *Neisseria meningitidis* or *Neisseria gonorrhoeae* species.

32. The attenuated mutant bacterium of claim 28, which has a mutation of a gene essential for production of a bacterial metabolite or catabolite not produced by a human or animal.

33. The attenuated mutant bacterium of claim 28, which has an attenuating mutation of a gene selected from *aro*, *asd*, *pur* and *pyr* genes.

34. The attenuated mutant bacterium of claim 33, wherein said mutation is of a gene selected from *aroA*, *aroB*, *aroC*, *aroD*, *aroL*, *purA*, *purB*, *purE*, *pyrA*, *pyrB* and *pyrE*.

35. The attenuated mutant bacterium of claim 28, which has a *recA* mutation.

36. The attenuated mutant bacterium of claim 28, which has a mutation by which expression of a toxin gene has been modified or eliminated.

37. The attenuated mutant bacterium of claim 28, which has a mutation at a site homologous to the *E. coli minB* locus.

38. The attenuated mutant bacterium of claim 28, which has a mutation in a gene involved in uptake of DNA.

39. The attenuated mutant bacterium of claim 38, which is of a species selected from *N. meningitidis* and *N. gonorrhoeae*, and wherein said mutation in said gene involved in uptake of DNA is a *comA* mutation.

40. The attenuated mutant bacterium of claim 28, which is of a species selected from *N. meningitidis* or *N. gonorrhoeae* and which has a mutation in the *galE* gene.

41. The attenuated mutant bacterium of claim 40, which further has a mutation in the *opc* gene to modify or eliminate expression of *opc* protein.

42. An attenuated mutant bacterial strain of the species *N. meningitidis* which has a genotype selected from:

- (a) mutation of *aroB*, *lac:fur* fusion, and mutation of *recA*;
- (b) mutation of *aroB*, mutation of *galE*, *lac:fur* fusion, and mutation of *recA*;
- (c) mutation of *aroL*, *lac:fur* fusion, and mutation of *recA*; and
- (d) mutation of *aroL*, mutation of *galE*, *lac:fur* fusion, and mutation of *recA*.

43. The attenuated mutant bacterial strain of the species *N. meningitidis*, according to claim 42, which also has at least one characteristic selected from: a *minB* mutation; an RTX negative phenotype; and an *opc* gene mutation whereby expression of said *opc* gene has been modified or eliminated.

44. A preparation of membrane vesicles obtained by isolating bacterial membrane vesicles from cultured cells of a mutant bacterium having a genome wherein a native *fur* gene having a function of ferric uptake regulation has been modified by mutation whereby expression of a gene product corresponding to said *fur* gene is regulated independently of the iron concentration in the environment of the bacterium.

45. A method of treating a subject which is a human or non-human animal, said method comprising vaccinating said subject with the vaccine composition of claim 25 thereby to stimulate an immune response against said bacterium.

46. A method of manufacturing a vaccine composition which comprises the attenuated mutant bacterium of claim 28, which process comprises:

- (a) inoculating said attenuated mutant bacterium into a culture vessel containing a nutrient medium suitable for growth of said bacterium;
- (b) culturing said bacterium;
- (c) recovering bacteria from the culture; and
- (d) mixing said bacteria with a pharmaceutically acceptable diluent or carrier.

47. A method of producing the attenuated mutant bacterium of claim 28, said method comprising introducing, into a genome of an attenuated bacterium, a mutation of a native bacterial *fur* gene having a function of ferric uptake regulation, whereby expression of a gene product corresponding to said *fur* gene is regulated independently of the iron concentration in the environment of the bacterium.